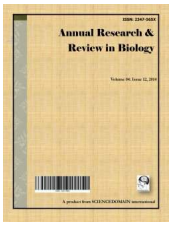

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Oxidative Damage and Reproductive Toxicity Associated with Antouka Super® in Male Japanese Quail (*Coturnix coturnix japonica)*: The Protective Effects of Hydroethanolic Leaves Extract of *Persea americana*

Ngoumtsop Victor Herman[1], Ngoula Ferdinand[1*], Ngouateu Kenfack Omer Bebe[2], Kenfack Augustave[1], Nguemmogne Tamdem Ghislaine[1], Mutwedu Valence[1], Tchoffo Herve[1], Azafack Kana Dorice[1], Deutcheu Nienga Sorelle[1] and Manjeli Yacouba[1]

[1]Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O.Box 222, Dschang, Cameroon.
[2]Department of Animal Physiology, Faculty of Sciences, University of Yaounde I, P.O.Box 812, Yaounde, Cameroon.

Authors' contributions

This work was carried out in collaboration between all authors. Authors NVH, NF and MY contributed substantially to conception and design of the study, data analysis and interpretation. Authors NVH, NF, NTG, MV, TH, AKD and DNS contributed in data acquisition. Authors NVH, NF and KA contributed in drafting the article or revising it critically for important intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

The present study was undertaken to evaluate the protective role of hydroethanolic leaves extract of The present study was undertaken to evaluate the protective role of Hydroethanolic leaves *Persea americana* (HEPA) against reproductive toxicity induced by Antouka Super® (AS) in male

**Corresponding author: E-mail: ferdinand.ngoula@univ-dschang.org, fngoula@yahoo.fr*

Japanese quail. The study was carried out in the Teaching and Research Farm of University of Dschang between February and May 2016. Forty (40) immature male Japanese quails (28 days old), were divided into five groups of 8 birds each and subjected to the following treatments: Group 1, birds receiving 10 ml/kg b.w of distilled water (negative control group (CO-); Group 2, birds receiving 75 mg of AS/kg (positive control group (CO+). while groups 3, 4 and 5 were administered 50, 100 and 200 mg/ kg b.w of HEPA respectively together with AS at 75 mg/kg. All the test solutions were orally administered once a day for 60 days using an endogastric canule. Dissection of the vas deferent was performed to obtain spermatozoa. The protective effects of HEPA on the organ weights, serum hormones, oxidative stress biomarkers, sperm characteristics and histology changes in the testes were evaluated. Results revealed that exposure to AS significantly ($p<0.05$) decreased reproductive organ weights (testes, epididymis and vas deferens); the levels of testicular proteins and of serum hormones (LH, FSH and Testosterone). This insecticide also significantly ($p<0.05$) decreased sperm characteristics (mobility, viability and density) and fertility indices (percentage of fertile eggs, hatching rate and chick survival rate after hatching), and increased the sperm abnormalities (minor and major) and the embryonic and post-embryonic mortality rate. In addition, the activities of superoxide dismutase (SOD), total peroxidase (POD) and catalase (CAT) significantly ($p<0.05$) decreased in the testes of AS treated quails. While the level of malondialdehyde (MDA) significantly ($p<0.05$) increased compared with the values recorded in the negative control group birds. Histopathological examination of the testes of AS treated quails revealed testicular lesions characterized by moderate to severe degenerative changes of seminiferous tubules and incomplete spermatogenesis. Administration of HEPA to treated birds alleviates the reproductive toxicity and testicular oxidative damage induced by AS. Thus, exposure of male Japanese quails to AS induce oxidative stress and impairment on the reproductive parameters. These effects can be mitigated by the administration of HEPA.

Keywords: Antouka Super® (AS); reproductive toxicity; oxidative damage; hydroethanolic leaves extract; Persea Americana (HEPA); Japanese quail.

1. INTRODUCTION

Pesticides have brought the green revolution in the world and are being widely used to control agriculture pests causing public health hazards including infertility. The infertility rate has increased tremendously in the past few decades [1,2]. The decline in sperm counts by about 50% may be the main cause of male infertility [3]. Exposure to chemical agents including pesticides has contributed to this decline [4]. Owing to the extensive use pesticides in agriculture, there is a high risk of organism exposure to these chemicals [5].

In fact, pesticides are known to increase the production of Reactive Oxygen Species (ROS), which in turn generate oxidative stress in different tissues [6]. Many studies have elucidated oxidative damage as the central mechanism of toxicity [7,8]. Oxidative damage primarily occurs through excessive production of ROS that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues [9,10]. Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipids bilayers [11] and they may damage membranes by inducing lipid peroxidation (LPO) [12,10].

Antouka Super® is a broad-spectrum insecticide widely used in agricultural and crops storage in many countries including Cameroon. It is made up of two insecticides: (Pirimiphos-methyl 16% and Permethrin 3%).

Pirimiphos-methyl is a broad-spectrum organophosphate insecticide that distresses the nervous system by inhibiting acetyl cholinesterase activity [13]. It is employed in agriculture to control insects and mites that affect cereals, fruits, stored grains and cotton. Ngoula et al. [14] reported that treatment of adult male rats with pirimiphos-methyl at the doses of (62.5-125) mg/kg b.w for 90 consecutive days is detrimental to the reproduction.

Permethrin is a pyrethroid insecticide class. It is an axonic poisons that affects nerve fibers by binding to a protein that regulates the voltage-gated sodium channel [15]. As pirimiphos-methyl, It is employed in agriculture to control insects and mites that affect cereals, fruits, stored grains

and cotton. Zhang et al. [16] showed that Permethrin dramatically reduces testosterone levels and sperm counts in adult male mouse.

In ours previous study, Antouka Super® (AS) increased the production of ROS which in turn increased lipid peroxidation and decreased the levels of oxidative stress biomarkers, such as superoxide dismutase (SOD), catalase (CAT) and total peroxidase (POD). This insecticide also decreased the sperm characteristics (mobility, viability and density), the level of serum testosterone and the fertility parameters (percentage of fertile eggs, hatching rate and chick survival rate after hatching). The contrary was recorded for the embryonic and post-embryonic mortality rate and the sperm anomalies (major and minor) (Personal communication).

As mechanism of AS toxicity involved oxidative stress, numerous efforts were done to identify dietary compound able to strengthen the cellular antioxidant defense so as to counteract the oxidative stress. In this respect, medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. More attention has been paid to the protective effects of natural antioxidants against ROS [17,18]. The increasing interest in the health properties of *Persea americana* and its main quercetin polyphenols have led to a significant rise in scientific investigation for prevention and therapeutics in several diseases [19]. Agomuo et al. [20] and Arukwe et al. [21] reported that the leaves of *Persea americana* displays antioxidants and free radical scavenger properties. Considerable data on the protective effects of plants extract against pesticides on some reproductive performances in mammal species are documented, but information related to birds are rare. The present study aims to investigate the protective effects of HEPA against AS induced reproductive toxicity and oxidative stress in male quail.

2. MATERIALS AND METHODS

2.1 Chemical

Antouka Super® (SYNGENTA, United Kingdom) is a combined insecticide whose active principles are Pirimiphos-methyl (0, 2-diethylamino-6-methylpirimidin-4-yl O, O-dimethyl phosphorothioate) concentrated at 19 g/kg and Permethrin (1*RS*, 3RS; 1*RS*, 3*SR*) - 3- (2, 2-Dichlorovinyl)- 2, 2- dimethylcyclopropane-1-carboxylate (3- phenoxyphenyl)) concentrated at 3 g/kg.

2.2 Plant Harvesting and Extract Preparation

Persea americana leaves were collected in October 2015 at Dschang, West Region of Cameroon and authenticated at the Cameroon National Herbarium under the voucher number 18604/Sfr/Cam. The plant material was dried on the shade, grinded to obtain fine powder which was macerated in the ethanol (70°) for 72 hrs. After filtration, the filtrate was concentrated under vacuum to remove ethanol and further dried using freezer dryer to obtain a fine powder.

2.3 Phytochemical Screening

Chemical screening of the HEPA revealed the presence of alkaloids, tanins, phytosterols, triterpenes, anthraquinones, phenols, saponines, flavonoïdes, glucosides and coumarin.

2.4 Birds

Healthy twenty-eight (28) days male Japanese quails weighing between 109 and 118 g were used in this study. Birds were housed in specialized wire cages, twelve per cage, in animal care facility, maintained at 22 to 25°C with a relative humidity of 76 ± 5%, for 7 weeks. Birds were kept in a 12 h light-dark cycle and had free access to water and a laboratory diet.

2.5 Experimental Design

Birds were randomly divided into five groups of 8 quails each and treated as follows: Group 1, birds receiving 10 ml/kg b.w of distilled water (negative control group (CO-); Group 2, birds receiving 75 mg of AS/kg b.w (positive control group (CO+). while groups 3, 4 and 5 were administered 50, 100 and 200 mg/ kg b.w of HEPA respectively together with AS at 75 mg/kg b.w. All the test solutions were administered *per os* once a day for 60 days using an endogastric canule. The doses of AS used in the study were selected from a pilot study and represent 1/15 of LD_{50} value obtained in quails (1125 mg/kg b.w) (personal communication). During the treatment, body weight was weekly measured.

2.6 Fertility Test

At the end of the treatment, male quails were allowed to mate (1:2) untreated proven fertile female quails. Mating was confirmed by the

presence of spermatozoa deposition in the vaginal orifice upon vaginal examination. On the other hand, eggs were collected 2 days after the observation of sperm cells in the vaginal orifice and for 7 days. Eggs were incubated for 19 days and unhatched eggs were opened and examined to see whether they were fertilized or not (presence of embryo or germinal disk). The male fertility parameters were calculated according to the following formulae:

Percentage of fertility eggs = (Number of fertile eggs/ Number of total incubated eggs) x 100

Hatching rate = (Number hatching eggs/ Number of incubated eggs) x 100

Embryonic mortality = (Number of dead chicks in eggs/ Number of total fertile eggs) x 100

Post-mortality embryonic= (Number of dead chicks/ Number of total chicks) x 100

Chicks viability at (14 days) = (Number of viable chicks/Number of total live chicks) x 100

2.7 Blood and Organ Collections

At the end of treatment (60^{th} day), blood was collected after sectioning the jugular vein of each bird. Serum was prepared and stored at -20℃ prior to analysis. After killing the quail, organs like testes, epididymis and vas deferens were carefully removed, free of adipose tissue, blotted dry and weighed separately. The left testes of each bird was then homogenized at 20% (weight/volume) in 0.9% NaCl solution and aliquots of supernatant were kept at - 20℃ prior to biochemical analysis.

2.8 Evaluation of Sperm Characteristics

Immediately after the sacrifice of each bird, vas deferens were carefully removed, minced in a 10 ml of 0.9% NaCl (40℃) and used to evaluate the sperm motility, concentration, viability and morphology. The sperm motility was estimated on scale basis as reported by Mamun et al. [22], sperms viability expressed as percentage of swelled sperms and the morphology expressed as percentage of abnormal sharp sperm. Sperm viability and morphology were analyzed using respectively hypo-osmotic swelling test Amorim et al. [23] and eosine/nigrosine test. Five microliters of sperm were mixed with 5 microliters of eosine/nigrosine solution. While 10 microliters of sperm were mixed with 1000 microliters of hypo-osmotic solution. The morphological defects of head, mid-piece, tail and the proportions of cells affected were evaluated. For each of the both parameters (viability and morphology), a total of 200 spermatozoa were counted in at least five different microscope fields according to the protocol provided by Revell and Mrod [24]. The sperm density was determined using Thoma hemocytometer.

2.9 Biochemical Analysis

The level of proteins in the testis was determined using CHRONOLAB kit following the manufacturer's protocol. Serum LH, testosterone and FSH levels were determined using appropriate commercially available kits (ELISA AccuDiag^{TM}, Diagnostic Automation Inc, 23963 Craftsman Rd Suites: D/E/F Calabas, ca 91305 USA and ELISA EIA, Gmrh, DRG, 1288 Germany). Total peroxidase (POD); superoxide dismutase (SOD); malondialdehyde (MDA) and catalase activity (CAT) were measured in testicular homogenates using spectrophotometer (GENESYS 20.0) and according to the methods described respectively by: Habbu et al. [25] and Dimo et al. [26] and Kodjo et al. [27] and Sajeeth et al. [28].

2.10 Tissue Preparation and Histopathology

The right testis was fixed in Bouin fluid for 1 week, embedded in paraffin, cut at 5 µm and stained with Harris hematoxylin and eosin. Tissue sections were observed under a light microscope (Leica DM 750, X40) for changes in the seminiferous tubules and intertubular spaces.

2.11 Statistical Analysis

Values are presented as Mean ± SEM. ANOVA was performed for comparison with post-hoc Duncan test to compare the level of significance between the control and experimental groups. A value of $p \leq 0.05$ was considered statistically significant. Statistical analyses were performed with the aid of SPSS for Windows software program (Release 20.0).

3. RESULTS AND DISCUSSION

3.1 Relative Weight of Some Reproductive Organs

The relative weight of testis, epididymis and vas deferens of birds in group CO+ was significantly

lower compared to the values of this parameter recorded in groups T1, T2, T3 and CO-. Furthermore, the relative weight of testis, epididymis and vas deferens of birds in groups treated with HEPA shown no significant differences when compared to group CO- (Table 1).

3.2 Oxidative Stress Biomarker

As recorded in Table 2, oral administration of AS at 75 mg/kg b.w for 60 consecutive days caused a significant decrease in the levels of proteins in the testes, and the activities of SOD, CAT and POD, as compared to the negative control (CO-). Co-administration of HEPA at different levels with 75 mg AS/kg b.w increased in a dose-dependent manner the values of all these oxidative stress parameters. The inverse was recorded for MDA concentration (Table 2).

3.3 Serum Levels of Reproductive Hormones

The levels of FSH, LH and Testosterone in different treatments are reported in (Table 3). Oral administration of AS at 75 mg/kg b.w induced a significant ($p<0.05$) decrease in serum FSH, LH and Testosterone concentration. HEPA administration significantly increased levels of theses hormones as compared to the positive control (CO+) group (Table 3).

Table 1. Effects of HEPA on body and reproductive organs weight of male Japanese quails exposed to AS

Weight parameters (mg)	Doses of HEPA (mg/kg b.w)				
	CO^- (n=8)	CO^+ (n=8)	T1 (n=8)	T 2 (n=8)	T3 (n=8)
Initial body	113.83±4.45	113.83±4.95	113.00±3.35	113.17±4.53	114.17±2.48
Final body	228.17±2.85^a	187.17±16.15^d	200.83±10.36^c	211.50±13.38^{bc}	219.00±7.92^{ab}
Body gain	110.64±14.29^a	71.00±14.95^c	87.83±10.96^b	98.33±17.00^{ab}	103.33±8.89^{ab}
Relative reproductive organs (%)					
Testis	1.54±0.14^a	0.54±0.19^c	1.24±0.18^b	1.31±0.12^b	1.35±0.18^{ab}
Epididymis	0.029±0.003^{ab}	0.023±0.008^b	0.031±0.004^a	0.031±0.005^a	0.028±0.007^{ab}
Vas deferens	0.037±0.008^{ab}	0.025±0.007^b	0.043±0.010^a	0.043±0.008^a	0.044±0.015^a

n=number of animals, each value represents mean ± standard error mean
(a,b,c,d) means bearing different letters in a row differ significantly at $p< 0.05$.
CO-: Negative control; CO+: 75 mg AS/kg b.w; T1: 75 mg AS+ 50 mg HEPA/kg b.w; T2: 75 mg AS+100 mg HEPA/kg b.w; T3: 75 mg AS+200 mg HEPA/kg b.w. HEPA: hydroethanolic leaves extract of Persea americana

Table 2. Effects of HEPA on oxidative stress biomarkers in the testis of male Japanese quail exposed to AS

Oxidative stress parameters in the testis	Doses of HEPA (mg/kg b.w)				
	CO- (n=8)	CO+ (n=8)	T1 (n=8)	T 2 (n=8)	T3 (n=8)
Testicular protein (mg/ml)	9.53±0.47^a	4.18± 0.11^e	5.45±0.31^d	6.36±0.27^c	8.15±0.37^b
MDA (nmol/mg tissues)	11.39±1.94^e	23.87±1.47^a	21.54±0.62^b	15.94±1.13^c	14.54±0.85^d
SOD (UI/mg tissues)	22.47±1.11^a	10,66±0,51^e	12.09±0.64^d	16.22±0.67^c	17.92±1.41^b
CAT (UI/mg tissues)	6.52±0.32^a	4.75±0.12^e	5.08±0.03^d	5.30±0.03^c	5.80±0.12^b
POD (µM/mg tissues)	19.02±0.40^a	12,93±0,33^c	13.61±0.45^c	14.95±0.70^b	18.39±1.23^a

n=number of animals, each value represents mean ±standard error mean
(a,b,c,d,e) means bearing different letters in a row differ significantly at $p< 0.05$.
MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase and POD: total peroxidase
AS: Antouka Super®; CO-: Negative control; CO+: 75 mg AS/kg b.w; T1: 75 mg AS+ 50 mg HEPA/kg b.w; T2: 75 mg AS+100 mg HEPA/kg b.w; T3: 75 mg AS+200 mg HEPA/kg b.w; HEPA: hydroethanolic leaves extract of Persea americana

Table 3. Effects of HEPA on serum hormones of male Japanese quails exposed to AS

Hormones characteristics (ng/ml)	Doses of HEPA (mg/kg b.w)				
	CO- (n=8)	CO+ (n=8)	T1 (n=8)	T 2 (n=8)	T3 (n=8)
FSH	4.08±0.37^{a}	1.35±0.18^{c}	1.56±0.10^{c}	3.00±0.77^{b}	3.35±0.27^{b}
LH	3.46±0.13^{a}	0.7±0.14^{e}	1.1±0.08^{d}	1.75±0.05^{c}	2.03±0.13^{b}
Testosterone	1.91±0.16^{a}	0.46±0.01^{d}	0.51±0.01^{cd}	0.6±0.02^{c}	1.14±0.06^{b}

n=number of animals, each value represents mean ±standard error mean
(a,b,c,d,e) means bearing different letters in a row differ significantly at p< 0.05.
FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone
AS: Antouka Super®; CO-: Negative control; CO+: 75 mg AS/kg b.w; T1: 75 mg AS+ 50 mg HEPA/kg b.w; T2: 75 mg AS+100 mg HEPA/kg b.w; T3: 75 mg AS+200 mg HEPA/kg b.w; HEPA: hydroethanolic leaves extract of Persea americana

3.4 Sperm Traits

The sperm motility and viability as well as the number of spermatozoa per vas deferens decreased significantly ($p<0.05$) in treated birds compared to negative control group birds (CO-) (Table 4). Generally, birds that were given AS together with HEPA had demonstrated a significant ($p<0.05$) increase in their sperm mobility, viability and density per vas deferens when compared to the positive control group (CO+) values. The inverse was observed with the major and minor abnormalities.

3.5 Fertility

The percentages of fertile eggs, hatching rate and chick survival 14 days after hatching in treated groups dropped significantly in intoxicated birds ($p<0.05$) compared to those of the negative control group (CO-) group. Co-administration of AS with HEPA significantly increased the values of these parameters (percentages of fertile eggs, hatching rate and chick survival 14 days after hatching). The inverse was recorded for the percentages of embryonic and post-embryonic mortality (Table 5).

3.6 Histological Findings

The testicular histology of treated and untreated quails are illustrated in Figure 1. Typical structure of testis was observed in control Japanese quails; the seminiferous epithelium contained all generations of germinal cells corresponding to the stages of seminiferous epithelium cycle. The lumen contained normal flagellated spermatozoa (1). In the second and third groups (2 and 3), more severe changes were revealed: dramatic depletion in the germ layers of seminiferous tubules with the degeneration of connective tissue between seminiferous tubules and increased intertubular space. While in the fourth and five groups (4 and 5), a slight degeneration in the germ layers of seminiferous tubules and intertubular space were observed. The lumen contained normal flagellated spermatozoa.

Table 4. Effects of HEPA on some sperm traits of male Japanese quails exposed to AS

Semen characteristics	Doses of HEPA (mg/kg b.w)				
	CO- (n=8)	CO+ (n=8)	T1 (n=8)	T 2 (n=8)	T3 (n=8)
Mobility (%)	4.16±0.51^{a}	2.41±0.66^{d}	2.83±0.40^{cd}	3,08±0.20^{bc}	3.50±0.63^{b}
Viability (%)	75.67±5.37^{a}	36.17±3.76^{d}	40.83±2.48^{cd}	45.33±9.07^{c}	64.67±5.35^{b}
Number/vas deferens (10^{9})	2.32±0.12^{a}	0.67±0.052^{d}	0.73±0.13^{d}	1.01±0.07^{c}	1.58±0.08^{b}
Semen morphology (%)					
Major anomalies	5.83±2.13^{c}	19.83±1.47^{a}	12.50±3.56^{b}	12.67±2.65^{b}	7.83±3.81^{c}
Minor anomalies	8.83±2.04^{d}	22.50±3.27^{a}	20.67±3.20^{ab}	17.50±3.93^{bc}	14.83±2.99^{c}

n=number of animals, each value represents mean ±standard error mean
(a,b,c,d) means bearing different letters in a row differ significantly at p< 0.05.
AS: Antouka Super®; CO-: Negative control; CO+: 75 mg AS/kg b.w; T1: 75 mg AS+ 50 mg HEPA/kg b.w; T2: 75 mg AS+100 mg HEPA/kg b.w; T3: 75 mg AS+200 mg HEPA/kg b.w; HEPA: hydroethanolic leaves extract of Persea americana

Table 5. Effects of HEPA on the fertility of male Japanese quails exposed to AS

Parameters	Doses of HEPA (mg/kg b.w)				
	CO- (n=8)	CO+ (n=8)	T1 (n=8)	T 2 (n=8)	T3 (n=8)
Total number of eggs	48	48	48	47	47
Fertile eggs (%)	89.58±4.50^{a}	50.00±4.50^{c}	67.80±4.50^{b}	66.66±4.50^{b}	72.53±4.50^{b}
Hatching rate (%)	91.06±5.23^{a}	76.25±5.23^{ab}	68.75±5.23^{b}	72.02±5.23^{b}	85.06±5.23^{ab}
Embryonic mortality (%)	8.93±5.23^{b}	23.75±5.23^{ab}	31.25±5.23^{a}	27.97±5.23^{a}	14.93±5.23^{ab}
Post-embryonic mortality (%)	20.55±5.40^{b}	42.08±5.40^{a}	41.66±5.40^{a}	26.66±5.40^{ab}	20.23±5.40^{b}
Viability of chicks at (14 days) (%)	79.44±5.40^{a}	57.91±5.40^{b}	58.33±5.40^{b}	73.33±5.40^{ab}	79.76±5.40^{a}

n=number of animals, each value represents mean ±standard error mean
(a,b,c,d) means bearing different letters in a row differ significantly at p< 0.05.
AS: Antouka Super®; CO-: Negative control; CO+: 75 mg AS/kg b.w; T1: 75 mg AS+ 50 mg HEPA/kg b.w; T2: 75 mg AS+100 mg HEPA/kg b.w; T3: 75 mg AS+200 mg HEPA/kg b.w; HEPA: hydroethanolic leaves extract of Persea americana

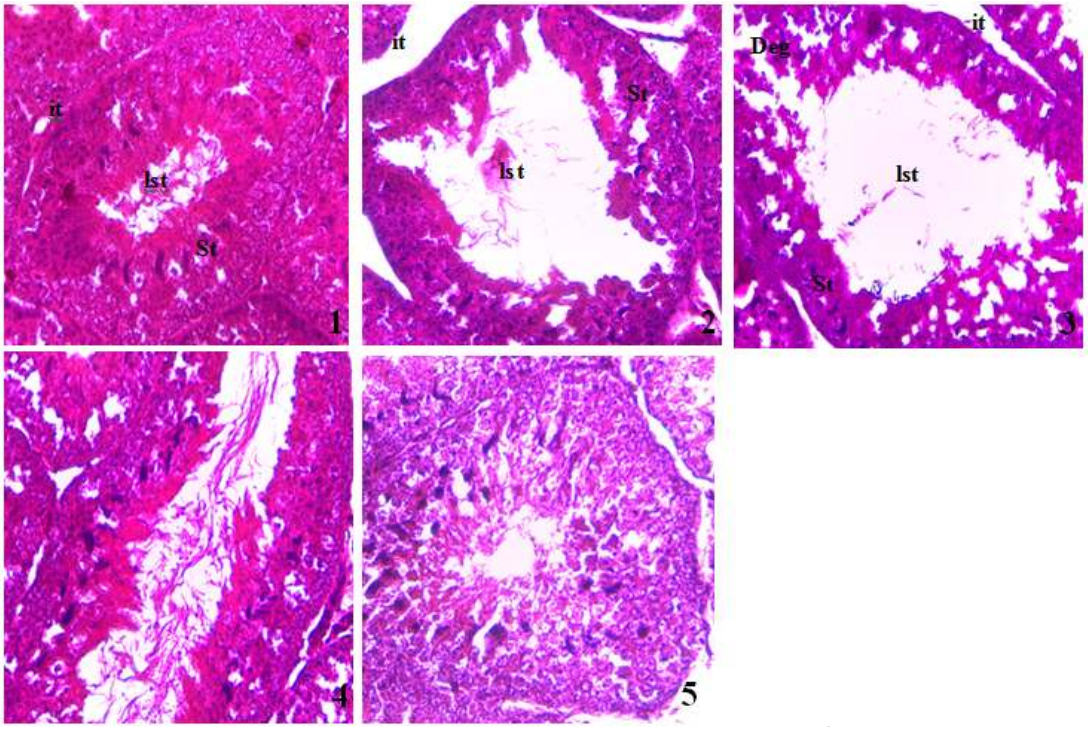

Figure 1. Micrographs of the section of quail testis (H&E X 400), 1: normal testis (control), showing normal structure with more spermatozoa in the lumen of seminiferous tubules (lst); 2:(75 mg AS/kg) showing dramatic depletion in the germ layers of seminiferous tubes (St) and degeneration of connective tissue between germ layers (Deg), seminiferous tubes are poor in spermatozoa (lst) and increased intertubular space (it); 3:(50 mg HEPA/kg) showing severe degeneration (Deg) and space formation in the germ layers of seminiferous tubes (St) the lumen of these seminiferous tubes also present less spermatozoa (lst) and increased intertubular space (it); 4:(100 mg HEPA/kg) showing slight degeneration in the germs layers of seminiferous tubules (Deg) and intertubular space (it), the lumen of the seminiferous tubes present more spermatozoa (lst) and 5:(200 mg HEPA/kg) showing slight degeneration in the germs layers of seminiferous tubules (Deg), lumen of the seminiferous tubes present more spermatozoa (lst)

4. DISCUSSION

The reproductive toxicity of Antouka Super® (AS) in Japanese male quail was characterized by a low fertility indices, a decrease of testes, epididymis and vas deferens weight, and low sperm characteristics such as sperms motility, viability and concentration. These findings are in agreement with those of [29,30] who reported reduction in fertility indices, a decrease of testes, epididymis and vas deferens weight, and low sperm characteristics such as sperms motility, viability and concentration after chronic exposure of male rats to Cypermethrin and Fenitrothion respectively. The reduction in the testicular weight reflects deleterious changes in seminiferous tubules. Since sperm motility is an important parameter to predict sperm fertilizing capacity, any negative impact on motility would seriously affect fertility. In this respect, marked inhibition of sperm motility in AS treated group may be due to a rapid loss of ATP, causing axonemal damage [31]. Full ATP pool is crucial for normal sperm movement and a slight deprivation of ATP leads to the reduction in sperm motility, which may cause infertility. Sperm density is considered as be one of the important factors affecting fertility [32,33] and the inhibition of gonadotrophins might have caused a decrease in sperm density [34]. Also, toxicants have direct effect on Sertoli cell function, which appears to be involved in the control of spermiation and when disturbed, caused epithelial disorganization, impaired spermatogenesis and subsequent tubular atrophy [35]. The decrease in sperm motility recorded in this study could be attributed in part to the sperm abnormalities and low viability. The decrease of the fertility parameters may be attributed to lack of forward progression and reduction in density of spermatozoa.

After exposure of male quail to AS for 60 days, a decrease in serum levels of FSH, LH and testosterone was recorded. These results are similar to those reported by [29] in mice exposed to Cypermethrin and by [36] in rats exposed to propoxur. In the present findings, reduction in the Testosterone, LH and FSH levels may be either due to the direct effect of AS on the androgen biosynthesis pathway in the testis or its effect on brain hypothalamus/anterior pituitary gland may be indirectly affecting the testes and sexual function and finally, the testosterone biosynthesis in the testes. The decrease of the testosterone level may also be induced by the stimulation of P450 aromatase (P450 arom), which catalyzes estrogen production from androgen; thereby decreasing androgen levels. These results suggest that AS exert suppressive effects on testicular function and impair fertility of male quail. Treatment of intoxicated quail with HEPA generally corrected the recorded damages.

The correction of reproductive damage in intoxicated male quails treated with HEPA can be due to the phytosterols, saponins, polyphenols and flavonoids present in the extract. Many studies have shown that these compounds increase the level of testosterone, the main hormone that controls sexual behavior [37,38,39].

A significant increase in the lipid peroxidation (LPO) level was observed in the present study. These results are in line with the observations of previous researchers after Cypermethrin (insecticide) administration [40]. Oxidative stress refers to disrupted redox equilibrium between the production of free radicals and the ability of cells to protect against damage caused by these species. The main cellular components susceptible to be damage by free radicals are lipids (peroxidation of unsaturated fatty acids in cell membrane); these free radicals can impair cellular structure and function [41,42]. It has been indicated that LPO is one of the molecular mechanism involved in pesticide-induced toxicity. Defense against oxidative stress are maintained using several mechanisms which include antioxidant machinery [43,44].

Reproductive toxicity could also be explained by the impaired antioxidant enzyme activities in the testes of the quails. This study also found a decrease of the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and total peroxidase (POD) after exposure of male quail to AS. Similar results were reported by [45,46,47]. These enzymes work together to eliminate active oxygen species. In this respect, SOD accelerates the dismutation of superoxide radicals (O^{2-}) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) while H_2O_2 is neutralized by the combined action of CAT and POD in all organisms [43,44]. POD is a group of antioxidant enzymes such as glutathione peroxidase (GPx) and glutathione S transferase (GST). The major function of these enzymes, which use glutathione as a substrate, is to reduce soluble hydrogen peroxide and alkyl peroxidases [48]. Pesticides have been reported to significantly decrease GPx and GST activities in male testicular tissues [48,10]. In the present

study, the drop of POD activities might reflect cellular oxidative stress due to pesticides exposure.

As regards to the histopathological results, testicular damage induced by AS exposure in intoxicated quails, as demonstrated in this study, is in agreement with that of many previous investigators who reported variable degrees of degenerative changes after exposure of male to insecticides [49,29]. Testicular damage induced by AS in this study confirms the reported lowered fertilizing capacity of the treated quails. The toxic effects of AS on male reproductive system of the Japanese quails could be explained by its direct cytotoxic effect and/or indirectly by the decrease serum hormones level.

Most of the biochemical alterations accompanied by histopathological changes were alleviated after administration of HEPA. This could be attributed to the antioxidant compound (polyphenols and flavonoids) found in this extract that reduces the LPO which in turn restore the integrity of the cell membrane and improve the disturbance in permeability. Since the oxidative damage as the central mechanism of pesticides toxicity occurs primarily through production of ROS, the use of antioxidants to counteract the formed ROS is the corner stone in alleviating such hazards. So, the major bioactive compounds in HEPA are the quercetin (polyphenol) that has the most effective antioxidant activity [19]. Quercetin is an efficient free radical scavenger due to their one electron reduction potential [50,19]. In addition, HEPA contains some co-factors of antioxidant enzymes: zinc, selenium, iron, vitamins (A, $B_{1,2,3}$, C et E) and manganese [20,21]. Polyphenols and flavonoids have additional mechanisms in which they reduce oxidation level through the inhibition of metal ions such as iron and copper and preventing their participation in oxidation reactions (leading to the formation of hydroxyl radical). Polyphenol and flavonoids also react through the suppression of oxidation stimulants such as xanthine oxidase and induction of antioxidant enzymes such as glutathione S-transferase and super oxide dismutase [51,52].

5. CONCLUSION

This study revealed that AS induces reproductive toxicity in male Japanese quail characterized by a decrease in the fertility indices, weights of sexual organs, some sperm characteristics and serum hormones level as well as testicular damage (induction of lipid peroxidation and depletion of antioxidant enzymes in testes). In contrast, treatment with HEPA inhibit the reproductive toxicity and oxidative damages induced by the insecticide. Finally, we can say that HEPA leaves may provide a cushion for prolonged therapeutic option against toxins-induced reproductive toxicity and oxidative damage without harmful side effects.

ETHICAL APPROVAL

Experimental protocols used in this study were strictly conformed with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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